American Journal of Hematology 52:1–7 (1996)

Random Suppression of T Cells That Bear Specific T Cell Receptor Vβ Sequences in Adult T Cell Leukemia/Lymphoma (ATLL) Patients at Each Clinical Stage: Carrier, Smoldering, Chronic, and Acute

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Human T cell leukemia virus type I (HTLV-I) is associated with adult T cell leukemia/lymphoma (ATLL), which is well known as a T cell malignancy. In order to clarify whether HTLV-I plays a role as a virus-encoded superantigen in the neoplastic process, we examined the TCR Vβ families in the peripheral blood at four different clinical stages: carrier, smoldering leukemia, chronic leukemia, and acute leukemia. An increased number of CD4 T cells was found in each of the four clinical stages. However, we found neither uniform specific losses nor uniform clonal expansion of particular TCR Vβ gene families in any case from the four clinical stages. However, a suppression of the random TCR Vβ families was found. Our data did not therefore directly suggest the existence of a common superantigen model of HTLV-I which induces an increase in CD4 T cells. The random suppression in the TCR Vβ repertoire is most likely caused by the influence of HTLV-I neoplastic pathogenesis rather than by virus-encoded superantigens. In the patients with acute leukemia, one or two families of the Vβ repertoires were very strongly expressed, while in chronic leukemia, no such repertoire of strong expression was observed. The immunological reaction of the hosts might thus be different between the above described groups. © 1996 Wiley-Liss, Inc.

Key words: adult T cell leukemia/lymphoma, T cell receptor Vβ, HTLV-I

INTRODUCTION

Adult T-cell leukemia/lymphoma (ATLL) is a human T-cell leukemia virus type I (HTLV-I) associated T-cell malignancy. Its characteristic clinicopathologic features include a rapidly progressing clinical course accompanied by the appearance of tumor cells with flower-like nuclei in the peripheral blood and a diffuse infiltration of various-sized neoplastic T cells with an irregular nuclear contour [1–3]. ATLL can be diagnosed by the clinicopathologic findings and the presence of integrated proviral HTLV-I DNA in the tumor cells with a helper/inducer (CD4) phenotype [4–6]. Clinically they are classified into four types: smoldering leukemia, chronic leukemia, acute leukemia, and lymphoma types [7].

The ability to characterize the T cell receptor (TCR) repertoire by identifying the individual variable (Vβ or Vα) gene families has provided a new approach for the analysis of immunological disorders, such as autoimmune and superantigen-mediated disease [8]. Superantigens are molecules, including endogenous retroviral gene products and microbial toxins, that can stimulate proliferation or, conversely, cause either clonal anergy or the deletion of a large percentage of T cells bearing a particular TCR Vβ sequence [9]. Recently, the issue of whether or not human immunodeficiency virus type I (HIV-I) encoded superantigens has generated much interest. Boldt-Houle et al. [10] reported the random depletion of TCR Vβ families.

Received for publication March 9, 1995; accepted August 9, 1995.

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To study the relationship between the usage of TCR Vβ and clinicopathologic features, we examined 32 ATLL patients who were identified as either being carriers, or smoldering, chronic, or acute leukemia cases.

MATERIALS AND METHODS

Cell Samples

Mononuclear cells of the peripheral blood (n = 32) were obtained from 32 ATLL patients, consisting of carriers, smoldering leukemia cases, chronic leukemia cases, and acute leukemia cases, who were clinicopathologically diagnosed from 1990 to 1994 at both Fukuoka and Kyushu Universities and their affiliated hospitals. The antibody against HTLV-I (adult T cell leukemia antibody, ATLA) was measured in the sera. The clinical data were obtained from the patient charts and their prognoses were provided by their physicians. The phenotype of the mononuclear cells in the peripheral blood cells was examined by monoclonal antibodies of CD2, 3, 4, 8, and/or 25 (Becton-Dickinson, Sunnyvale, CA), using a FACScan analyzer (Becton Deckinson).

Southern Blot Analysis

A part of the frozen material was used for DNA isolation and a gene analysis. The details of the examination methods have been previously reported [11]. The proviral DNA of HTLV-I (full length; gag, pol, env, pX, LTR) was examined by a Southern blot analysis. DNA was digested with either restriction enzyme EcoRI or PstI to analyze the monoclonal or polyclonal integration of HTLV-I. T-cell receptor (TCR) genes Cβ, Jγ, and immunoglobulin heavy chain (JH) were examined for the monoclonal population.

Polymerase Chain Reaction (PCR)

Isolated DNA was used for the PCR with specific primers synthesized on the basis of the published DNA sequence. For HTLV-I, primer pX-1 and pX-2 were synthesized, corresponding to the pX region of HTLV-I [12]. An HTLV-I full-length probe was used. Amplification was done with the GeneAmp DNA amplification reagent kit and DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, CT). To exclude any nonspecific reaction, we added perfect-match polymerase enhancer (Stratagene, La Jolla, CA). After 30 cycles of PCR amplification, we analyzed one-tenth of the reaction mixture (10 μL) by Southern blot methods, using an HTLV-I probe (Oncor, Gaithersburg, MD). The oligo-primers and probes are summarized in Table I.

Reverse Transcription PCR (RT-PCR)

The total RNA was prepared from frozen materials and PBMCs by the guanidium thiocyanate-phenol-chloroform method using a Total RNA Separator kit (Clontech Laboratories, Palo Alto, CA). We used 250 ng of the RNA preparation for RT-PCR (Thermostable rTth Reverse Transcriptase RNA PCR kit, Perkin-Elmer Cetus). The primers for the amplification of the TCR β chain V regions were primer Vβ1, Vβ2, Vβ3, Vβ4, Vβ5.1, Vβ5.2, Vβ6, Vβ7, Vβ8, Vβ9, Vβ10, Vβ11, Vβ12, Vβ13.1, Vβ13.2, Vβ14, Vβ15, Vβ16, Vβ17, Vβ18, Vβ19, Vβ20, and Cβ3′, synthesized on the basis of a published report [13]. After 35 cycles of PCR amplification, we analyzed one-tenth of the reaction mixture (10 μL) by Southern blot methods using a Cβ5′ probe. To detect HTLV-I tax1/rex1 mRNA, we synthesized primer RPX3 and RPX4, based on the findings of a previous report [14].

TABLE I. Primers and Probes for Polymerase Chain Reaction

Primer/Probe		5′ → 3′ Sequence
pX1	(primer)	ATGCTGTTTCGCCTTCTCAG
pX2	(primer)	TAAGGACCTTGAGGGTCTTA
RPX3	(primer)	ATCCCGTGGAGACTCCTCAA
RPX4	(primer)	AACACGTAGACTGGGTATCC
Vβ1	(primer)	GCACAACAGTTCCCTGACTTGCAC
Vβ2	(primer)	TCATCAACCATGCAAGCCTGACCT
Vβ3	(primer)	GTCTCTAGAGAGAAGAAGGAGCGC
Vβ4	(primer)	ACATATGAGAGTGGATTTGTCATT
Vβ5.1	(primer)	ATACTTCAGTGAGACACAGAGAAAC
Vβ5.2	(primer)	TTCCCTAACTATAGCTCTGAGCTG
Vβ6	(primer)	AGGCCTGAGGGATCCGTCTC
Vβ7	(primer)	CCTGAATGCCCCAACAGCTCTC
Vβ8	(primer)	ATTTACTTTAACAACAACGTTCCG
Vβ9	(primer)	CCTAAATCTCCAGACAAAGCTCAC
Vβ10	(primer)	CTCCAAAAACTCATCCTGTACCTT
Vβ11	(primer)	TCAACAGTCTCCAGAATAAGGACG
Vβ12	(primer)	AAAGGAGAAGTCTCAGAT
Vβ13.1	(primer)	CAAGGAGAAGTCCCCAAT
Vβ13.2	(primer)	GGTGAGGGTACAACTGCC
Vβ14	(primer)	GTCTCTCGAAAAGAGAAGAGGAAT
Vβ15	(primer)	AGTGTCTCTCGACAGGCACAGGCT
Vβ16	(primer)	AAAGAGTCTAAACAGGATGAGTCC
Vβ17	(primer)	CAGATAGTAAATGACTTTCAG
Vβ18	(primer)	GATGAGTCAGGAATGCCAAAGGAA
Vβ19	(primer)	CAATGCCCCAAGAACGCACCCTGC
Vβ20	(primer)	AGCTCTGAGGTGCCCCAGAATCTC
Cβ3′	(primer)	GTGCACCTCCTTCCCATT
Cβ5′	(probe)	GTGTTTGAGCCATCAGAA

RESULT

Clinical Features

We selected 32 patients, including HTLV-I carriers, smoldering leukemia cases, chronic leukemia cases, and acute leukemia of ATLL cases, who were all clinicopathologically diagnosed. All patients were positive for an anti-HTLV-I antibody. The acute type exhibited increased atypical flower-like ATL cells in the peripheral blood, and showed a poor prognosis with elevated serum lactic dehydrogenase (LDH). The chronic type exhibited in-

TABLE II. Clinical Data

Case no.	Age (yrs)	Sex	Diagnosis	WBC (mm^3)	Atypical lymphocytes (%)	CD4 (%)	CD8 (%)	(4/8)	CD25 (%)	HTLV-I Eco/Pst	PCR	RT-PCR	TCR
1	68	F	Carrier	4,800	1	63	30	2.1	1.0	−/−	+	−	G
2	50	F	Carrier	5,200	2	/	/	/	/	−/−	+	−	/
3	48	M	Carrier	4,200	0	/	/	/	/	−/−	+	−	G
4	71	M	Carrier	4,000	0	47	43	1.1	/	−/−	−	−	G
5	44	M	Carrier	10,000	1.5	63	30	2.1	0.7	−/−	+	−	G
6	40	M	Carrier	9,500	0	50	34	1.5	/	−/−	+	+	G
7	45	F	Carrier	3,500	0	33	33	1.0	2.7	−/−	−	−	G
8	67	M	Carrier (HAM)	8,300	0	51	49	1.2	0.4	−/−	+	+	G
9	62	M	Smoldering	6,900	6	51	42	1.2	1.4	−/−	+	−	/
10	54	F	Smoldering	24,200	4	30	53	0.6	/	+/+	+	+	G
11	41	M	Smoldering	12,200	1	52	43	1.2	4.9	+/+	+	−	R
12	57	M	Smoldering	9,500	9	69	32	2.2	/	−/+	+	−	G
13	74	F	Smoldering	7,820	8	40	11	3.6	20.1	−/−	+	−	G
14	59	M	Smoldering	11,170	25	43	50	0.9	4.5	−/+	+	+	/
15	5	M	Smoldering	6,500	5	63	39	1.6	7.6	−/−	+	+	G
16	41	M	Smoldering	12,000	7	53	27	2.0	1.2	+/+	+	+	R
17	42	F	Chronic	32,400	79	60	16	4.3	5.2	+/+	+	+	R
18	63	M	Chronic	15,500	11	72	21	3.4	1.4	+/+	+	+	R
19	45	M	Chronic	72,000	95	99	2	49.5	92.6	+/+	+	−	R
20	67	M	Chronic	18,000	30	97	6	16.2	/	+/+	+	+	/
21	41	M	Chronic	10,000	10	49	35	1.4	17.0	+/+	+	+	R
22	46	F	Chronic	29,500	54	94	10	9.4	/	+/+	+	+	R
23	51	F	Chronic	48,600	62	96	11	8.7	28.7	+/+	+	+	R
24	62	F	Chronic	8,000	8	80	11	7.3	12.6	+/+	+	+	/
25	56	F	Acute	44,600	24	10	3	3.3	50.7	+/+	+	+	R
26	71	F	Acute	20,900	88	91	44	2.2	1.3	+/+	+	+	R
27	64	M	Acute	21,700	44	96	2	48.0	2.9	+/+	+	+	R
28	69	M	Acute	40,000	60	99	2	49.5	20.3	+/+	+	+	R
29	59	M	Acute	16,000	50	99	89	1.1	20.9	+/+	+	+	/
30	78	F	Acute	14,600	74	96	87	1.1	60.2	+/+	+	−	/
31	76	M	Acute	7,500	25	80	18	4.4	/	+/+	+	−	R
32	61	M	Acute	66,000	84	97	1	97.0	28.5	+/+	+	−	R

creased WBC counts and elevated LDH, but the disease course was not as short as the acute type. The smoldering type showed the presence of a few atypical cells, and the serum LDH was within the normal limits. In addition, all patients were healthy and not on any other therapy. The carriers showed a few atypical cells in the peripheral blood in the time course (Table II).

HTLV-I and TCR Gene Analysis

To investigate the monoclonal proliferation of HTLV-I integrated lymphocytes, we used the restriction enzyme EcoRI; while for polyclonal integration, we used PstI and EcoRI. Monoclonal proliferation was found in all patients with chronic and acute leukemia, and in three of eight cases with smoldering leukemia, but in none of the carriers. Polyclonal proliferation was found only in two of eight patients with smoldering leukemia. However, the monoclonal bands of smoldering cases were fainter than those with acute and chronic leukemia, probably because of the small population of HTLV-I integrated lymphocytes.

After 30 cycles of PCR amplification, we performed a Southern blot analysis with an HTLV-I probe. An amplification of the HTLV-I pX regions was observed in all patients with acute, chronic, and smoldering leukemia, while we could not detect any amplified HTLV-I genes in two of the eight carriers, which was probably because the population of HTLV-I integrated lymphocytes was too small to detect the HTLV-I gene using PCR. In addition, no amplification band was detected in the control DNA from the placenta or peripheral blood of the people without serum-ATLA.

In the RT-PCR, mRNA of the HTLV-I tax/rex mRNA was detected in five of eight patients with acute leukemia, seven of eight chronic cases, four of eight smoldering cases, and two of eight carriers.

All cases of acute and chronic leukemia examined showed rearrangements of TCR C β and/or J γ, while four of six smoldering leukemia cases and no carriers showed such a rearrangement. In addition, no cases displayed a rearrangement of the immunoglobulin heavy chain gene (JH).

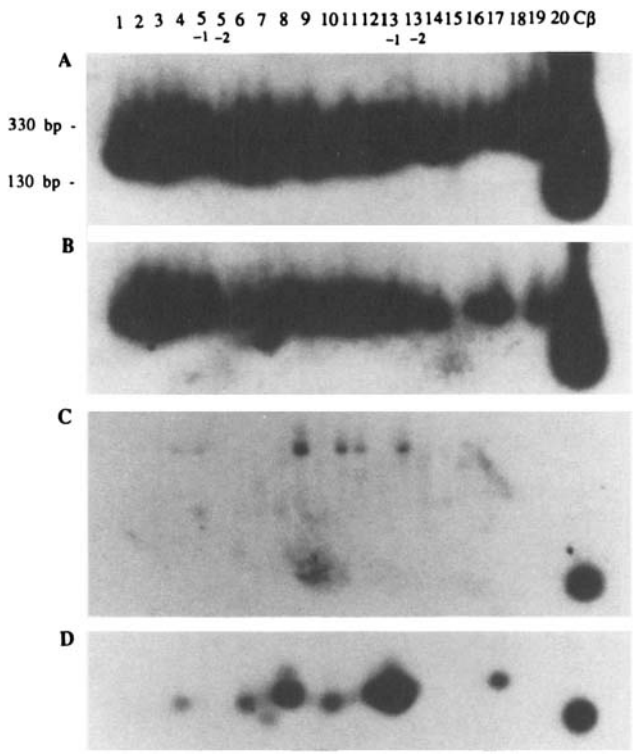

Fig. 1. TCR Vβ repertoire. Autoradiographic exposures of the RT-PCR amplified TCR Vβ and Cβ mRNA transcripts obtained from PBMCs, isolated from healthy, HTLV-I sero-negative controls (A), smoldering leukemia (B, case 12), chronic leukemia (C, case 19) and acute leukemia (D, case 31).

Surface Marker

The phenotype of the mononuclear cells in the peripheral blood cells was examined by a flow cytometric analysis.

The CD4/CD8 ratio increased in three of six carriers, four of eight smoldering leukemia cases, all chronic leukemia cases, and six of eight acute leukemia cases. In all patients with chronic leukemia and six of eight with acute leukemia, the phenotype of leukemic cells was CD4+ CD8− while in two of eight with acute leukemia, the phenotype was CD4+ CD8+.

The CD25-positive cells increased in two of six smoldering cases, four of six chronic cases, five of seven acute leukemia cases and in none of the carriers.

Expression of TCR Vβ

The Vβ repertoire of the mononuclear cells in the peripheral blood cells was analyzed using ^{32}P-labeled Cβ oligonucleotide. Representative autoradiograms are shown in Figure 1. In all three cases, who were HTLV-I sero-negative, all TCR Vβ repertoires were expressed.

In four of eight carriers, and one of eight smoldering cases, all TCR Vβ repertoires were expressed. All four types, including the carriers, smoldering cases, chronic cases, and acute leukemia cases demonstrated some diminishment of the Vβ repertoires, but the patterns of diminished repertoires were nonspecific. The number of the diminished TCR Vβ families was two to 14 (average; 3.1) in the patients with carrier, two to 19 (9.6) in smoldering, five to 19 (15.6) in chronic, and five to 20 (12.9) in acute. The number of the randomly deleted repertoires increased from the carriers to the smoldering, chronic, and acute leukemia cases. However, the number of patients with acute leukemia was higher than the smoldering cases, but was lower than those with chronic leukemia.

The relative signal strength for a given Vβ family varied between individuals. However, in the patients with acute leukemia, one or two families of the Vβ repertoire were expressed very strongly, in comparison with Cβ. While in the chronic leukemia cases, no such strong expression of the Vβ repertoires, like that seen in the acute leukemia cases was observed.

Importantly, no evidence of profound deletion unique to any Vβ-bearing T cells was found for any carriers, smoldering cases, chronic cases, or acute leukemia cases, despite their increased CD4 to CD8 ratio and the expression of HTLV-I tax/rex mRNA (Table III).

DISCUSSION

ATLL is a T cell malignancy derived from CD4 positive T cells with integrated proviral DNA of HTLV-I [1–6]. Some ATLL patients demonstrate a prodromal peculiar clinical manifestation before the onset of overt typical features. The clinical stage gradually progresses from carrier to the smoldering, chronic, and acute-type leukemia stage [7]. The model of HTLV-I proviral DNA integration also changes from undetectable to polyclonal, and then to monoclonal malignant transformation [15]. An analysis of the proviral DNA integrated in cellular DNA revealed that the leukemic cells were always infected with HTLV-I. These cells were also always monoclonal in respect to the proviral integration, which indicated that they originated from a single cell infected with HTLV-I [5]. In this study, the analysis of HTLV-I proviral DNA at the four clinical stages also showed the changes from undetectable to polyclonal, and then to monoclonal integration.

In contrast to other animal retroviruses, the HTLV-I genome has an extra sequence, the so-called pX region. The two genes identified in the pX region encode the trans-acting factors of tax and rex and these regions either activate or suppress the gene expression and replication of HTLV-I [12]. Tax is also a trans-activator for the transcription of some cellular genes such as interleukin-2 (IL-2), interleukin-2 receptor (IL-2R) α, parathyroid hormone

TABLE III. Expression TCR Vβ

Case no. TCR Vβ	1	2	3	4	5-1	5-2	6	7	8	9	10	11	12	13-1	13-2	14	15	16	17	18	19	20	Depletion
Carrier																							
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
5	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	−	−	−	+	−	−	+	7
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	2
7	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	2
8	−	+	+	+	−	−	+	−	+	−	−	+	+	−	−	−	−	−	+	−	−	−	14
Usage	7	8	8	8	6	6	8	7	8	7	7	7	8	7	7	5	5	6	7	6	6	7	
Smoldering																							
9	−	+	+	+	−	−	+	+	−	+	−	+	−	−	−	−	−	−	−	−	−	−	15
10	+	+	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	+	−	−	15
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	2
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	3
14	−	−	+	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	18
15	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	+	−	19
16	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	5
Usage	5	6	6	6	4	3	6	7	5	6	4	5	4	4	4	5	3	4	3	4	3	2	
Chronic																							
17	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−	+	5
18	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	19
19	−	−	−	+	+	−	−	−	−	+	−	+	+	−	+	−	−	−	−	−	−	−	16
20	−	−	−	+	+	−	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	16
21	+	+	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	18
22	−	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	19
23	−	+	−	−	+	−	+	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	17
24	−	+	+	+	+	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	15
Usage	2	5	3	5	7	0	2	3	2	5	2	3	2	1	5	2	0	0	1	0	1	1	
Acute																							
25	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	20
26	−	−	−	−	−	−	−	−	+	−	+	+	−	−	−	−	−	−	+	−	−	−	18
27	−	−	+	+	−	+[a]	−	+	−	+	−	−	+	+	−	+	−	−	+	−	−	−	13
28	−	+[a]	+	+	−	−	+	+	+[a]	+	+	+	+	+	−	+	+	−	+	−	−	−	8
29	−	−	+[a]	−	−	−	+	−	+	−	−	−	+	−	−	−	−	−	+	−	−	−	15
30	+	+	+	+	+	−	+	+	+	+[a]	+	+	+	+	−	+	−	+	+	+	−	−	5
31	−	−	−	+	−	−	+	+	+	−	+	−	+	+	−	−	−	−	+	−	−	−	14
32	−	−	+	+[a]	−	−	+	−	+	+	+	+	+	−	+	+	−	−	+	−	−	+	10
Usage	1	2	5	5	1	1	5	4	6	4	5	4	6	4	1	5	1	2	7	1	0	1	

[a]Strong expression.

related protein, c-fos, and major histocompatibility complex (MHC) class I [16,17]. In the early stage of leukemogenesis, it is suspected that the tax gene expression induces a multiclonal expansion of some subpopulations of the virus-infected T cells by an autocrine mechanism (that is, by the trans-activation of IL-2 and IL-2R by $p40^{tax}$) [18]. In fact, normal peripheral blood lymphocytes can be immortalized by HTLV-I. However, the expression of tax/rex cannot be detected by conventional methods [19]. They were detected in the peripheral blood mononuclear cells (PBMCs) of ATLL patients and asymptomatic HTLV-I carriers by using only very sensitive methods of RT-PCR. However, the amounts of detected mRNA corresponded to 10^4 to 10^6 times less than that found in the HTLV-I infected MT-2 cell line [14]. The level of tax/rex mRNA expression thus does not correlate with either the amount of atypical lymphocytes in the peripheral blood or the titer of antibodies against HTLV-I. These findings are also consistent with the idea that the expression of one or more HTLV-I gene may be involved in an initiating transformation but a consistent expression is not needed for the leukemogenesis of immortalized cells [14]. In addition, according to our study, the expression of the tax1/rex1 was not associated with the clinical stage, the expression of IL-2R (CD25) or the specific Vβ families.

The TCR on most peripheral T cells is composed of two proteins called α and β chains. Both chains have variable (V), joining (J), and constant (C) regions. During T cell development these genes are rearranged to form the

functional genes. The functional chains were composed of the specific Vβ and Vα segments, selected from a relatively small pool of about twenty families [20,21]. Some Vβ-specific antigens are endogenously synthesized, while some bacterial proteins have also been reported to possess this property [22,23]. The mode of action of these molecules has provided a new approach for the analysis of immunological disorders, such as autoimmune and superantigen-mediated disease [8]. The present study supports the hypothesis that the T-cell population is restricted through TCR Vβ expression.

The gradual decline of CD4+ T lymphocytes in HIV-infected individuals culminates in the lethal immunosuppression of AIDS. The mechanism of CD4+ T cell loss has recently been suggested to occur as a result of an HIV-encoded superantigen. Imberti et al. [24] reported that the selective elimination of T cells which express a defined set of Vβ sequences may also indicate the presence of an HIV encoded superantigen. But Boyer et al. [25] failed to provide evidence of a selective loss of Vβ-bearing T cells. In addition, Boldt-Houle et al. [10] also reported the random depletion of TCR Vβ families. These data suggest that CD4 loss in HIV patients does not occur in a Vβ-selective, superantigen-mediated fashion.

In our study, like HIV-I infection, the random depletion of TCR Vβ was found in the HTLV-I infection. The number of the depleted families increased from the carriers to the smoldering leukemia, chronic leukemia, and acute leukemia cases. Our data are therefore not directly suggestive of a common superantigen model of HTLV-I induced CD4 T cell increasing. The random suppression in the TCR Vβ repertoire is most likely due to HTLV-I neoplastic pathogenesis rather than virus-encoded superantigens. In the patients with acute leukemia, one or two families of Vβ repertoires were expressed very strongly, while, in chronic leukemia, no such repertoires of the strong expression were observed. The immunological reaction of the hosts might therefore differ between those groups.

Kimata et al. [26] investigated the possibility that HTLV-I may encode a superantigen specific for the TCR Vβ8 chain in vitro. But, it does not appear that HTLV-I encodes a superantigen specific for the TCR Vβ8 molecule. In our in vivo study, HTLV-I did not appear to play a role as a superantigen.

Previous studies using the monoclonal antibodies for TCR V regions (Vβ5 and 8) in T cell lymphoma have implied that Vβ gene use is random [27]. In addition, in the study using RT-PCR, only one or two of 20 Vβ families were distinguished in T-cell lymphoma, whereas some of the other Vβ families showed only a weak expression [28]. In the study of cutaneous T cell lymphoma (CTCL), 10 of 16 cases reacted with the monoclonal antibody specific for the Vβ8 gene family [29]. However, no cases of eczematoid or premycotic CTCL showed uniform reactivity with Vβ8. The non-selected reaction in the pre-neoplastic state probably means the processes to select one subpopulation by oncogenic transformation [29]. In our study, six of eight acute leukemia cases expressed strongly one or two Vβ families, while chronic leukemia cases only demonstrated a very weak expression of some restricted families. In other words, acute leukemic cells expressed one or two Vβ families, but chronic leukemic cells did not express Vβ families but instead suppressed them. We could not elucidate how chronic leukemic cells obtained the expression of Vβ families in the disease processes, because we could not perform the analysis in the followed case, where acute leukemia developed from a chronic leukemia.

Cytotoxic T lymphocytes (CTL) derived from the patient with HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) expressed CD8 antigen, and their function was restricted by HLA-A2 [30]. The cytotoxic effects appeared to be predominantly against the target cells expressing HTLV-I $p40^{tax}$ [31]. In our HAM/TSP case, the tax/rex expression was observed and TCR Vβ families were restricted. It might be due to the specific CTL population. But we could not obtain the experimental data supporting the specific CTL population.

ACKNOWLEDGMENT

This research was supported in part by grants-in-aid for the Encouragement of Young Scientists from the Ministry of Education, Science, and Culture, Japan.

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